

# Effects of oxygen concentration on microbial growth in aerated palm oil mill effluent using oxygen enriched air membrane system

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## Abstract

Aeration is one of the most important factors in biological wastewater treatment process. Through aeration, oxygen is supplied to the bacteria to allow their respiration to proceed rapidly and satisfy the biochemical oxygen demand (BOD). This study was carried out to determine the biological growth in aerated palm oil mill effluent (POME) when subjected to aeration using air of different oxygen concentration. Pure oxygen (101% O<sub>2</sub>), diffused air (20.9% O<sub>2</sub>), and oxygen-enriched air (26.7% O<sub>2</sub>) were used to aerate the POME for three weeks continuously at a flow rate of 0.1 LPM. Oxygen enriched air was supplied by an oxygen enriched air system (OEAS). This system is able to enriched oxygen concentration of air up to 26.7% using membrane with composition consisting of 22% polysulfone, 31.8% dimethylacetamide, and 14.4% ethanol. Samples subjected to aeration with air of three different oxygen concentration were tested for several water quality parameters which include pH, temperature, BOD, chemical oxygen demand (COD), dissolved oxygen (DO), total solid (TS), total suspended solid (TSS), bacterial count, saturated dissolved oxygen (SDO) and dissolved oxygen deficit for every two days. It was found that bacteria count and BOD reduction in POME when aerated by OEAS was 13.8% and 18.3% respectively more than when it was aerated by diffused air. This shows that an increased in oxygen concentration during aeration can speed up the microbial growth and thus shortened the period of aerobic digestion process.

*Keywords:* aeration, oxygen enriched air, oxygen transfer, bacterial count, water quality

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## 1.0 Introduction

Malaysia is the world's largest producer and exporter of palm oil with a share of 48% of world palm oil production and 60% of exports. When combined with palm kernel oil and oleochemical exports, Malaysia becomes the world's largest exporter of oils and fats products supplying 30% of total export. These achievements reflect the competitiveness and sustainability of palm oil production in Malaysia (Basiron, 2000). However, palm oil industry is also identified as the single largest source of water pollution in Malaysia.

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Several treatment methods have been successfully employed by the palm oil mills operators to treat their effluents before discharging into watercourses. The system includes waste stabilization pond (85% of the palm oil mills in Malaysia), anaerobic and aerobic digester, open tank digester and extended aeration, close tank digester with biogas recovery and land application, and thermophilic anaerobic contact process (Maheswaran, 1982 ). Concurrently, Muhamad (2000) reported that POME discharge need to be improved from time to time based on research findings. The first generation standard had been enforced in 1978. Since then POME discharge standard had been improved. To date the sixth generation standard is still in use (Muhamad, 2000).

Several studies had been conducted on the application of tertiary treatment on POME. Wong et al. (2003) studied on coagulation, centrifugation and filtration as the pretreatment before POME was treated using ultrafiltration membrane. It was found that filtration-ultrafiltration was the best combination to improve the final quality of POME. COD was reduced from 1336 mg/L to about 200 mg/L. Tajuddin et al. (2003) studied the effect of oxygen concentration in air used during aeration process of POME. It was found that oxygen enriched air improved the dissolved oxygen concentration in POME and thus improve the biological process during aerobic digestion. As such, sufficient oxygen supply is required for effective aerobic digestion to take place. Many studies have been conducted on aeration which involves the form of aeration methods, oxygen transfers rate, bubble formation and size, water and wastewater characteristics when subjected to aeration and many more.

Loubière et al. (2003) studied the effects of liquid cross-flow on the bubble formation at a single flexible orifice. Several forces acting on the bubble have been modeled in order to understand the dynamics of the bubble growth detachment. The experimental analysis of bubble growth has shown that, under liquid cross-flow conditions, the bubbles move downstream and are flattened during their growth (position of the bubble centre of gravity, bubble inclination angle). The bubble spread over the orifice surface, and the advancing and the receding bubble angles were measured. The detached bubbles have significantly smaller sizes and higher frequencies when compared to bubble formation under quiescent liquid conditions (Loubière et al., 2003).

However, Iranpour et al. (2002) carried out a case study of aeration performance under changing process conditions. Off gas analyses of oxygen transfer efficiency (OTE) at Terminal Island Treatment Plant of Los angeles document changing performance of fine-pore diffusers in an activated sludge plant from 1991 to 1998. The analysis of measurements showed the effects expected from fouling, and there was also extensive deterioration of the air distribution system. These measurements show the degree to which efficiency losses may grow unrecognized if OTE measurements are not done (Iranpour et al., 2002). In addition, Meric et al. (2003) investigated on an OUR-based approach to determine the toxic effects of 2,4-dichlorophenoxyacetic acid in activated sludge. This study uses the oxygen uptake rate (OUR) measurement to measure toxicity effects of 2,4-dichlorophenoxyacetic acid (2,4-D) on activated sludges fed with the wastewater from a small domestic wastewater treatment plant and peptone-based synthetic wastewater. It was noted that the OUR was decreased to 15 and 30%

respectively, when 75 mg l<sup>-1</sup> of 2,4-D was added to domestic and synthetic reactors. Meanwhile the addition of 25 plus 50 mg l<sup>-1</sup> of 2,4-D in sequence to domestic reactor did not significantly affect the OUR profile (Meric et al., 2003).

On the other hand, the effects of impurities in wastewater on oxygen transfer rate have been studied and contradictory results have been reported. Zieminski et al. (1967) studied the behavior of air bubbles in dilute aqueous solution and found that the volumetric mass-transfer coefficient increased in the presence of mono- and di-carboxylic acid and aliphatic alcohol. Zieminski and Lessard (1969) studied the effects of chemical additives on the performance of an air-water contactor and attributed the increase of the oxygen transfer rate in the increase of surface area. Koide et al. (1976) studied the mass transfer from single bubbles in aqueous solution containing surfactants and reported the volumetric mass-transfer coefficient decreased in the presence of surfactants. Kawase and Ulbrecht (1982) studied the effect of surfactant on the terminal velocity of gas bubbles and mass-transfer rate in a non-Newtonian fluid and found that the bubble terminal velocity and volumetric mass-transfer coefficient both decreased in the presence of surfactant. Gurol and Nekouinai (1985) studied the effect of organic substances on the mass-transfer rate in bubble aeration and reported the volumetric mass-transfer coefficient was decreased by the surface active compound, but increased by the alcohol and carboxylic acids. Wagner and Pöpel (1996) studied the effect of surface-active agents on oxygen transfer rate in a fine bubble aeration system and found that the volumetric mass-transfer coefficient decreased in the presence of surfactant. Wend et al. (1997) studied the effect of soybean oil on the oxygen transfer in penicillin production and found that soybean oil acted as a good foam inhibitor but decreased the oxygen transfer rate. Leu et al. (1998) studied the effects of surfactants and suspended solids on oxygen transfer rate and found that the volumetric mass-transfer coefficient decreased with increasing surfactant or suspended solid concentration to a minimum and then increased as the impurity concentration increased.

In this study, the effect of oxygen concentrations that was used during aeration on oxygen transfer and microorganism's population in POME was investigated. American Society of Engineers (1992) oxygen mass transfer model was adopted to evaluate the effect of oxygen concentration during aeration on the volumetric mass-transfer coefficient.

### *1.1. Oxygen transfer in wastewater*

Oxygen transfer or aeration plays an important role in biological wastewater treatment processes (Chern, 2000). Many different types of aeration systems have been developed over the years to improve the energy efficiency of oxygen transfer process. In order to evaluate the performance of different types of aeration systems, the ASCE and US EPA jointly developed a standard for the measurement of oxygen transfer in clean or tap water (ASCE, 1984). The standard conditions recommended by ASCE is zero dissolved oxygen level, 20°C water temperature, and 1 atm. pressure. The oxygen transfer rate at non-standard conditions is adjusted by the so called alpha, beta, and theta factors which depend on the types of aerators, mixing or turbulence intensity, and wastewater

characteristics, etc. (Gilbert, 1979; Stentrom and Gilbert, 1981; Doyle and Boyle, 1985; Eckenfelder, 1989; Asselin et al., 1998).

With the exception of certain industrial wastes, such as POME, wastewaters provide an ideal growth medium for a large number of different microorganisms, and microorganisms play a key role in all stages of biological wastewater treatments (Akunna et al., 2001). The removal of dissolved and particulate carbonaceous BOD and the stabilization of organic matter found in wastewater are accomplished biologically using a variety of microorganisms, principally bacteria. Microorganisms are used to oxidized or convert the dissolved and particulate carbonaceous organic matter into simple end products and additional biomass (Tchobanoglous et. al., 2003). An abundant oxygen supply in wastewater pond system is the key to rapid and effective wastewater treatment. Oxygen or oxygen enriched air is needed by the bacteria to allow their respiration reactions to proceed rapidly and satisfy the biochemical oxygen demand in biological treatment processes or to act as an agent in the oxidation of undesirable contaminant. Microorganisms have a number of vital functions in pollution control and especially in wastewater treatment. It is the microbial component of aquatic ecosystems that provides the self-purification capacity of natural waters in which organisms respond to organic pollution by increased growth and metabolisms (Hammer, 1986). Thus, the successful design and operation of wastewater treatment for aerobic digestion, requires an understanding of the types of microorganisms involved, the specific reactions that they perform, the environmental factors that affect their performance, their nutritional needs and their reaction kinetics which can be illustrated by Equation 1 and Figure 1.

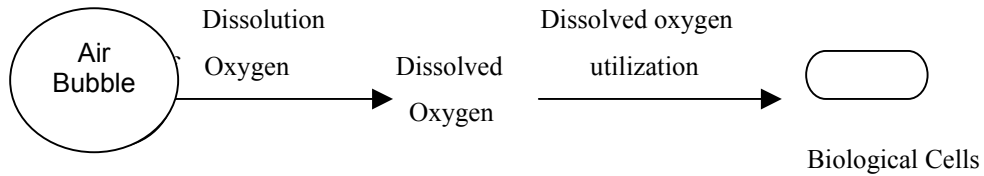
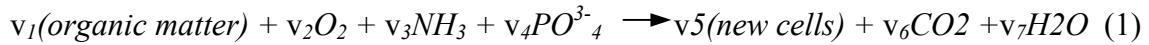


Figure 1 : Two-phase oxygen transfer in wastewater aeration (Metcalf & Eddy, 2003)

The following simplified mass transfer model were used to analyze the aeration data obtained:

- (a) To estimate the flux of a slightly soluble gas from the gas to the liquid phase (liquid film controls transfer rate, based on Fick's law) :

$$r = K_L(C_s - C_t) \quad (2)$$

where  $r$  is the rate of mass transferred per unit area per unit time ( $ML^{-2}T^{-1}$ ),  $K_L$  is the overall liquid mass transfer coefficient ( $LT^{-1}$ ),  $C_s$  is the concentration in equilibrium with gas as given by Henry's law ( $ML^{-3}$ ) and  $C_t$  is the concentration liquid bulk at time  $t$  ( $ML^{-3}$ ).

- (b) The rate of mass transfer per unit volume per unit time is obtained by multiplying equation (2) by the area A and dividing by the volume V:

$$r_v = K_L \frac{A}{V} (C_s - C_t) = K_L a (C_s - C_t) \quad (3)$$

where  $r_v$  is the rate of mass transfer per unit volume per unit time,  $\text{ML}^{-3}\text{T}^{-1}$ ,  $K_L a$  is the volumetric mass transfer coefficient,  $\text{T}^{-1}$ , A is the area through which the mass is transferred,  $\text{L}^2$ , V is the volume in which constituent concentration is increasing,  $\text{L}^3$  and a is the interfacial area for mass transfer per unit volume,  $\text{A/V}$ ,  $\text{L}^{-2}$ .

- (c) To determine the volumetric mass transfer coefficient ( $K_L a$ ):

$$\frac{dC}{dt} = K_L a (C_s - C_t) \quad (4)$$

Integrating equation 2 between the limits of  $C = C_o$  and  $C = C_t$  and  $t = 0$  and  $t = t$ , where  $C_o$  is the initial concentration and  $C_t$  is the concentration at some time t :

$$\int_{C_o}^{C_t} \frac{dC}{C_s - C_t} = K_L a \int_0^t dt \quad (5)$$

yields

$$\frac{C_s - C_t}{C_s - C_o} = e^{-(K_L a)t} \quad (6)$$

$$\ln \left( \frac{C_s - C_t}{C_s - C_o} \right) = -(K_L a)t \quad (7)$$

the terms  $(C_s - C_t)$  represents the degree of undersaturation at any time t and the term  $(C_s - C_o)$  represents the initial degree of undersaturation.

## 2.0 Experiment

Experiments were conducted at several phases. Phase one was sampling, followed by aeration process using the newly developed reactor and finally wastewater samples characterization before and after aeration.

### 2.1. Sampling

Samples were taken by grab sampling from the facultative pond effluent at Bukit Besar Palm Oil mill, Kulai, Johor, Malaysia. Characteristics of the samples are shown in Table 1:

Table 1

Initial characteristics of POME taken from facultative pond

Dissolved Oxygen (DO), mg/L	2.20
Biochemical Oxygen Demand (BOD), mg/L	189
Chemical Oxygen Demand (COD), mg/L	2873
Total solid (TS), x 10 <sup>2</sup> mg/L	3.91
Total suspended solid (TSS), x 10 <sup>2</sup> mg/L	1.73
Bacteria count, ( x 10 <sup>4</sup> CFU/ 100ml)	7
pH	8.41
Temperature	27
Saturated Dissolved Oxygen, mg/L	7.95

## 2.2. Aeration Process

The aeration system was designed as continuous flow system because air is continuously supplied into the aeration tank. For each aeration tank, pure oxygen (PO), oxygen enriched air (OEA), and diffused air (DA) was directly injected into the wastewater with air diffuser at the bottom of the tank. Three seven litre samples were placed in three separate containers. The wastewater was then aerated with PO; OEA and DA at a rate of 0.1 LPM simultaneously for three weeks to establish the growth medium for microorganisms. Routine laboratory measurements were conducted for every two days. These wastewater samples were tested for their initial characteristic which involves parameters such as BOD, COD, DO, TS, pH, temperature and bacteria counts before and after being subjected to the three modes of aeration mentioned earlier. Aeration set up is shown in Figure 2. PO, OEA and DA was supplied from pure oxygen tank, oxygen enriched air membrane system as shown in Figure 3 and compressed air. Summary of the experimental conditions were shown in Table 2:

Table 2

Summary of the experiment conditions

Pure oxygen concentration,	101%
Oxygen enriched air oxygen concentration	26.7%
Diffused air oxygen concentration	20.9%
Volume of samples, V; m <sup>3</sup>	7 x 10 <sup>-3</sup>
Aeration tank cross-sectional area, A; m <sup>2</sup>	0.0314
Flow rate of air supply, LPM	0.1
Hollow fiber membrane for oxygen-enriched air supply system was developed from dope composition	22% polysulfone 31.8% N,N dimethylacetamide 14.4% ethanol

The typical schematic arrangement of system's components shown in Figure 2 consists of air compression section, pretreatment section, air separation section and wastewater aeration section. The overall system was installed with the standard measuring instruments and equipments, and the whole system was using join stainless steel fittings and tubing. The fabricated oxygen enrichment system is very easy to operate and suitable for lab scale testing. In addition, this system is applicable to low purity oxygen enrichment applications (Ismail et al., 2003). Ambient air was used as a feed gas for air separation. A Puma Compressor (1) was used to compress the ambient air into the system. Air was typically compressed to pressures in the order of 155.14 cm Hg gauge to 517.15 cm Hg gauge (30 to 100 psig). Pressure ranges were controlled by a Koganei regulator (3). The compressed air was passed through several pre-treatment parts, such as air filter (4), carbon dioxide trap (5), and moisture trap (6), where to remove undesired substances such as large particles, compressor oil, carbon dioxide and moisture from the air feed stream (2). The undesired substances were extracted from the system in order to avoid product purity being contaminated. In addition, this undesired product could affect the system's operation conditions (Kiong, 2003).

### *2.3. Wastewater samples characterization and bacteria count*

Samples were characterized based on Standard Methods (Standard Methods, 1992). NWRI agar (HPCA) has been used to produce higher colony counts and incubated at 20°C for 7 days. Material used to produced nutrient agar (composition of nutrient agar): 3g peptone; 0.5g soluble casein; 0.2g potassium permanganate ( $K_2HPO_4$ ); 0.05 magnesium sulfate ( $MgSO_4$ ); 0.001g ferric chloride ( $FeCl_3$ ); 15g agar; and 1 L reagent-grade water.

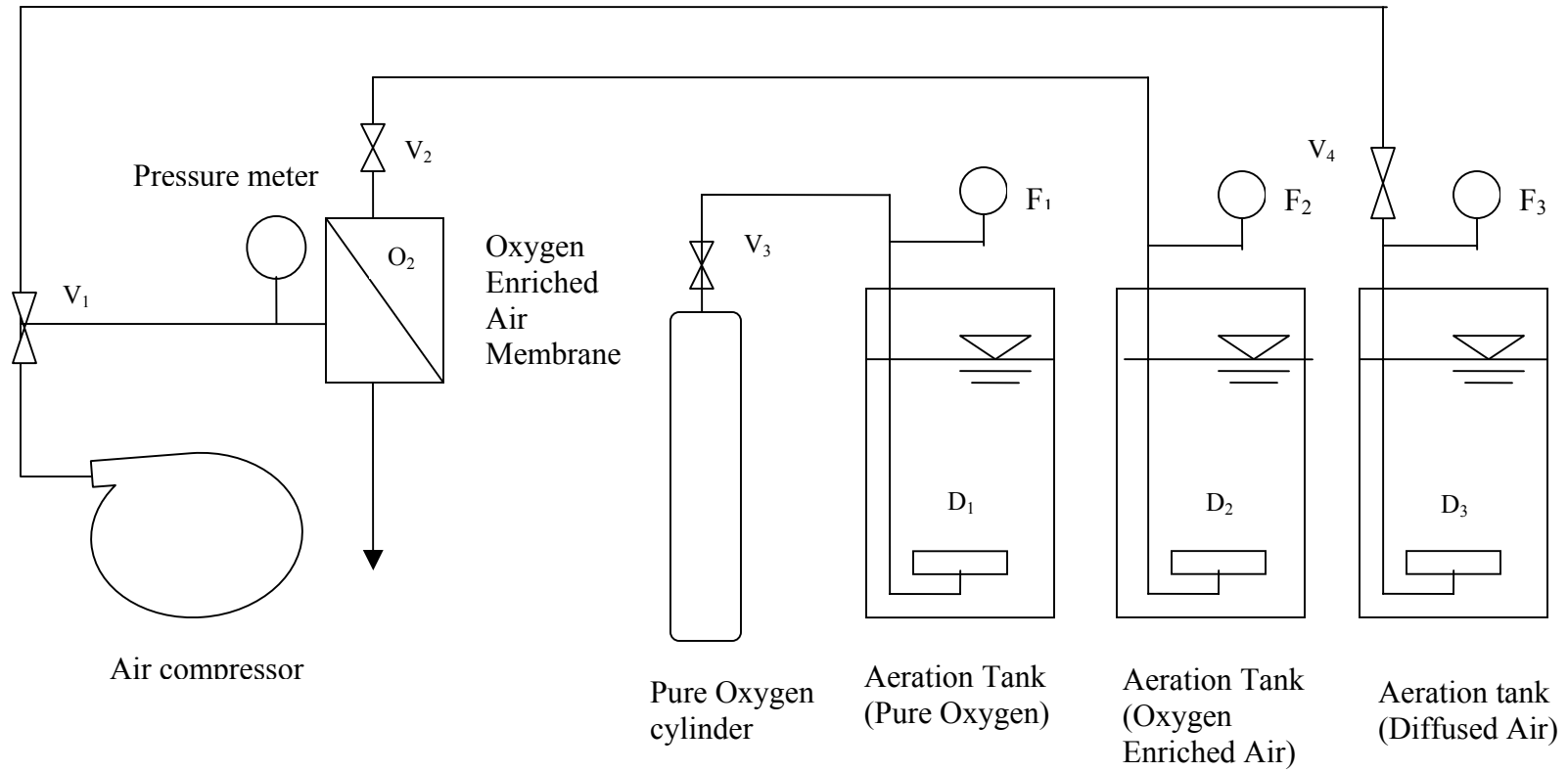


Figure 2: Aeration system set-up.  $V_1$  is valve controlling compressed air into Oxygen Enriched system.  $V_2$ ,  $V_3$  and  $V_4$  are valves controlling the flow of pure oxygen, oxygen enriched air and diffused air respectively.  $D_1$ ,  $D_2$  and  $D_3$  are diffusers through which the airs were supplied.



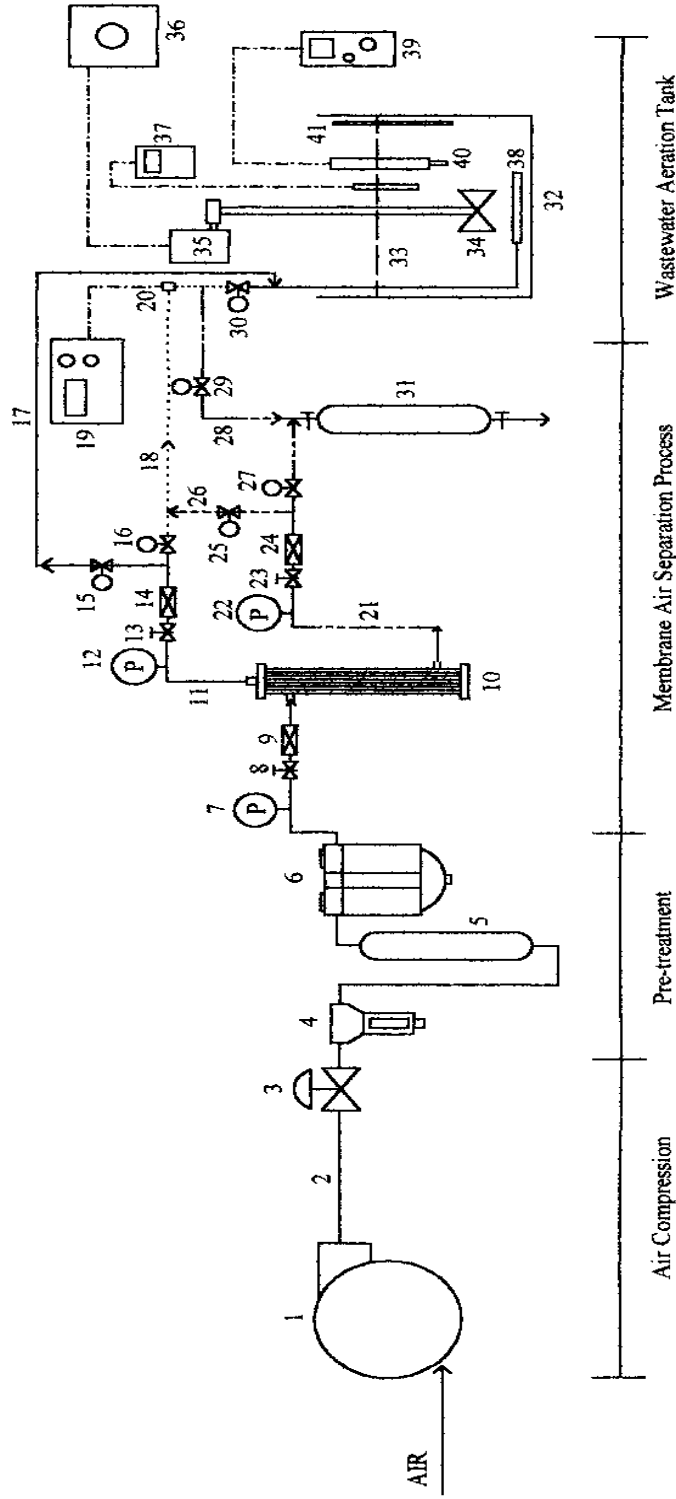


Figure 3: Schematic diagram of combination membranes oxygen enrichment system and wastewater aeration tank.

(1) Compressor; (2) Air feed stream; (3) Air regulator; (4) Air filter; (5) Carbon dioxide trap; (6) Moisture trap; (7) Feed pressure gauge; (8) Feed flow control valve; (9) Feed flow indicator; (10) Hollow fiber membrane module; (11) Permeate stream 1; (12) Permeate pressure gauge; (13) Permeate flow control valve; (14) Permeate flow indicator; (15, 16) Ball valve; (17) Permeate stream 2; (18) Share way; (19) Oxygen Analyzer; (20) Oxygen membrane probe; (21) Retentate pressure gauge; (22) Retentate pressure gauge; (23) Retentate flow control valve; (24) Retentate flow indicator; (25) Ball Valve; (26) Retentate stream 2; (27) Ball valve; (28) Retentate stream 3; (29, 30) Ball valve; (31) Sampling cylinder; (32) Wastewater aeration tank; (33) Wastewater; (34) Stirrer; (35) Motor; (36) Motor controller; (37) pH meter; (38) Diffuser; (39) Dissolved oxygen meter; (40) Dissolved oxygen probe; (41) Thermometer.

(Kiong, C. F.; 2002)

### 3. Results and discussion

#### 3.1. Effect of oxygen concentration on volumetric mass transfer coefficient ( $K_La$ , $T^{-1}$ )

Figure 4 (a) showed the increasing trend in  $K_La$  value from day zero to day two in all reactors indicating the dissolution process of oxygen in POME (Metcalf & Eddy, 2003). Bacteria activity was minimal since the numbers of bacteria in the reactor were only  $7 \times 10^4$  CFU/100ml at the initial stage. The curves dropped from day 2 to day 5 and approximately flattened thereafter can be explained through bacterial activity. The bacteria start to consume the DO and causes dropped in  $K_La$  value. As nutrients in the reactor (batch reactor) decreases, the bacterial activity slowed down and stabilized, and thus stabilizes the  $K_La$  values too.

Figure 4 (b) showed that oxygen concentration in the air supplied during aeration process influenced the  $K_La$  values in POME. It can be seen that PO gives the highest  $K_La$  value followed by OEA and DA. A similar phenomenon was experienced by Terasaka and Shibaka (2003) in their study on oxygen transfer in viscous non-Newtonian liquids yield stress in bubble columns. They found that the  $K_La$  increases with the increased in air velocity which reflects the volume of oxygen being supplied per unit time. In addition, the profile obtained in Figure 4(b) was in consistent with the trend determined by Maier et al. (2003) when oxygen transfer rate was plotted against dissolved oxygen concentration. Thus, it can be said that the oxygen transfer rate is governed by the DO concentration, and it applies also to POME.

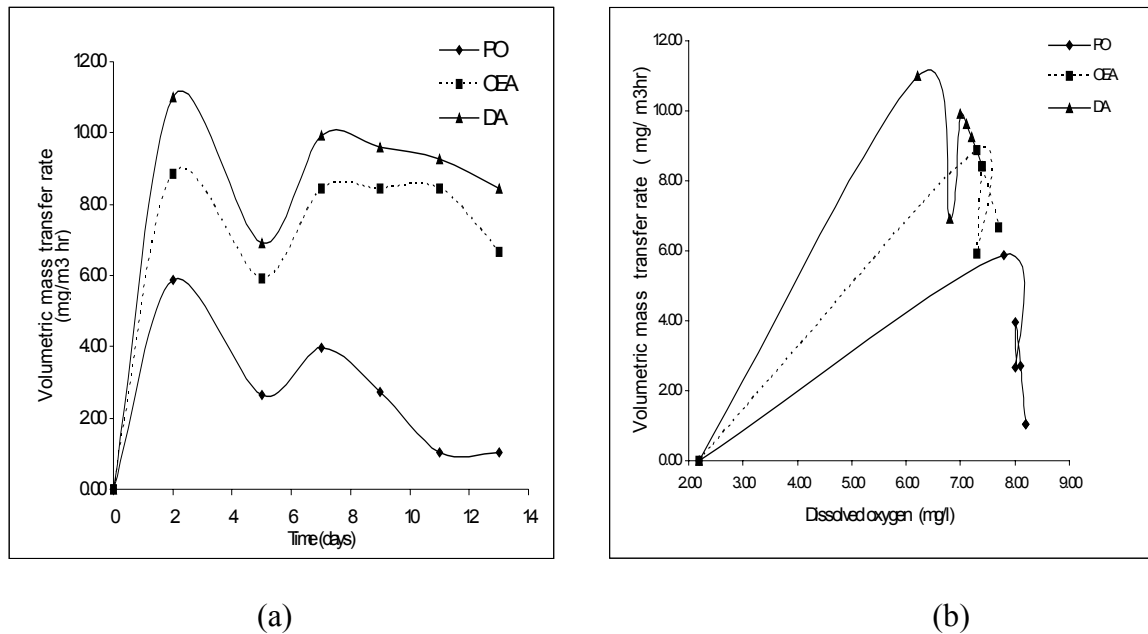


Figure 4: (a) showed the volumetric mass transfer of air at different oxygen concentration in POME during aeration period of 13 days and (b) volumetric mass transfer at different dissolved oxygen concentration during aeration process.

### 3.2. Effect of oxygen concentration on Oxygen Transfer Rate, Volumetric mass transfer coefficient and bacteria count.

Changing the operating conditions may affect the oxygen transfer rate (Maier et al., 2003) and if the metabolism of the organism under investigation remains unaffected by the oxygen limitation, the observed growth rate is a function of the mass transfer rate alone and gives no information about the true growth rate of the microorganisms as demonstrated by Van Suijdan et al. (1978). The same phenomenon was applied in this experiment, whereby oxygen was supplied continuously at different concentration for thirteen days. Figure 5(a) reveals that as the number of bacteria increases the volume of mass transfer also increases and PO transferred the highest volume, followed by OEA and DA. A sharp increase in volume of mass transfer was observed in the three different oxygen concentration aeration processes. This showed the oxygen deficit was high at the early stage of aeration. As aeration time increased, number of bacteria also increases and at the same time the volume of mass (oxygen) transfer increases but it was not as rapid as compared to the early stage. The volume of oxygen consumed by bacteria and the volume of oxygen transferred is moving towards stabilization. This can be seen in Figure 5(a), where the volume of oxygen transfer tend to stabilized when the bacteria count was about  $10 \times 10^4$  CFU/100ml.

On the contrary, the rate of mass transfer in DA showed the highest level which is followed by OEA and PO. The rate of oxygen transfer was rapid at the early stage of aeration process but declined later. Figure 5(b) revealed that rate of oxygen transfer does not depend on the number of bacteria but is based on oxygen deficit. As aeration time increases, the oxygen deficit decreases and thus the rate of oxygen transfer decreases.

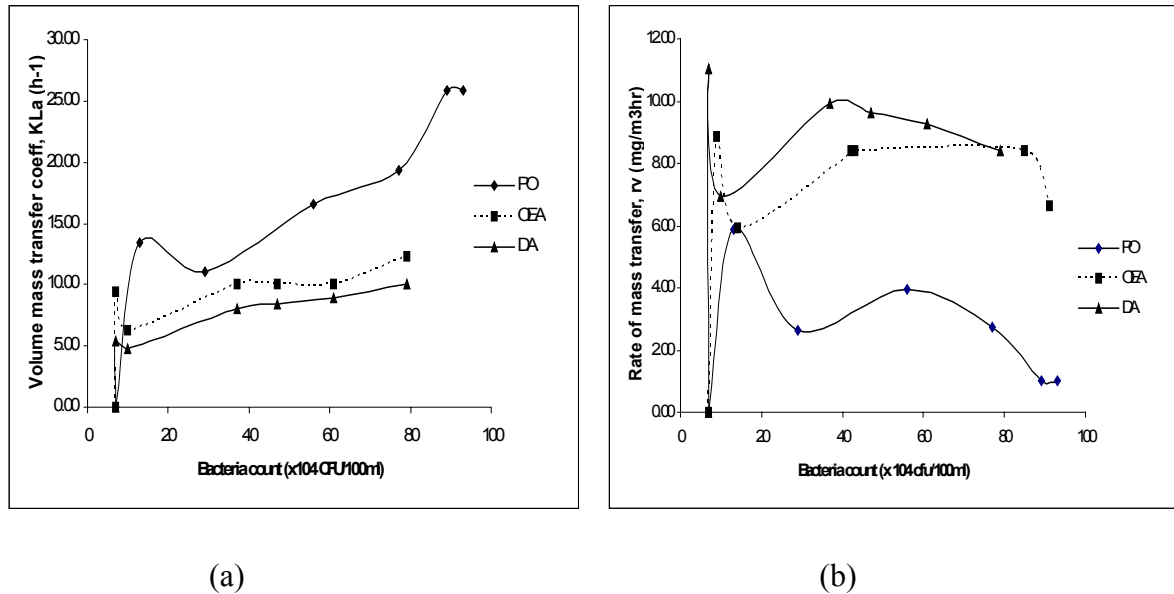


Figure 5 : (a) showed the effect of bacteria count on the volume mass transfer ( $K_L a$ ) of oxygen and (b) rate of oxygen transfer ( $r_v$ ) respectively.

### 3.2. Relationship of bacteria count with chemical oxygen demand (COD) removals during aeration

The COD was an important factor to evaluate the organic content in POME. The effect of aeration using air of different oxygen concentration on the reduction of COD was illustrated in Figure 6. COD in wastewater tank will increase in the absence of organisms (Karge and Pamukoglu, 2003). It implies that, the presence of organisms and their number plays a vital role in the process of reduction of COD, thus purifying wastewater. Figure 6 showed that as aeration time being prolonged, the number of bacteria increases and at the same time causes the reduction in COD concentration of POME.

Bacteria population growth is influenced by the dissolved oxygen concentration available (McGhee, 1991; Metcalf & Eddy, 2003; Hammer, 1986). Figure 6(a) and (b) showed the increase in population growth of bacteria when aeration was supplied continuously. During aeration, dissolved oxygen deficit was enriched resulting to the reduction in percentage oxygen deficit and at the same time enhancing the bacterial growth thus reducing the COD concentration. It was observed that the percentage oxygen deficit was smallest when aerated using PO (0.49%) followed by OEA (6.55%) and DA (10.19%).

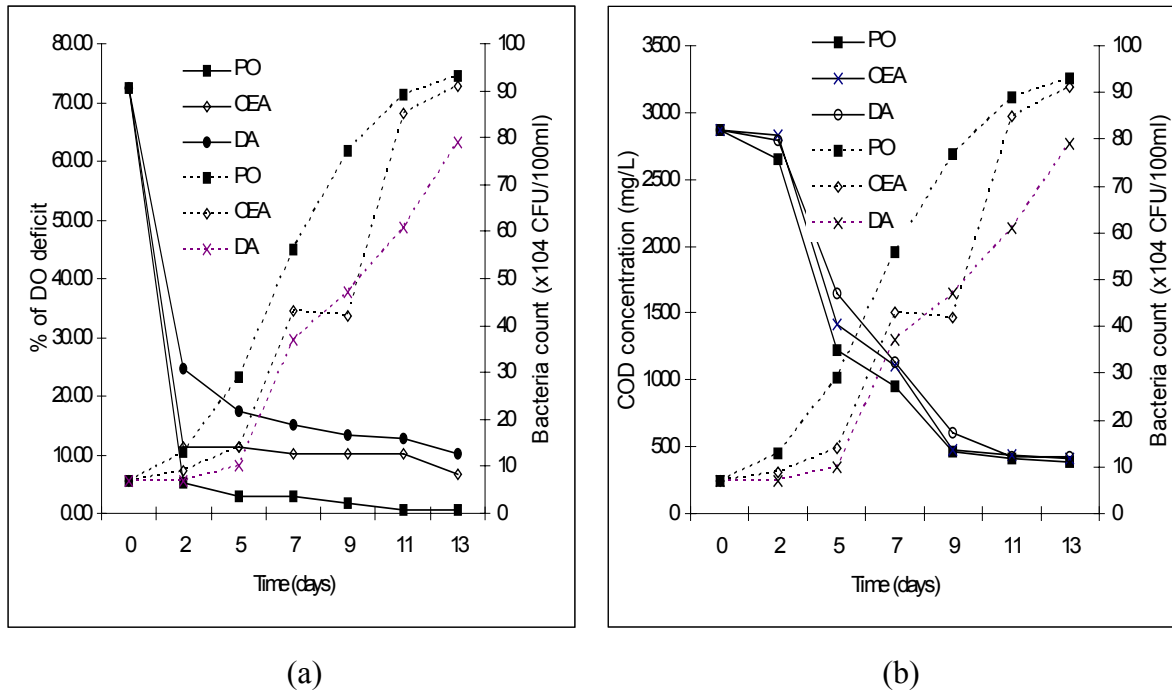


Figure 6: (a) Effect of oxygen concentration on % of DO deficit and bacterial growth and (b) Effect of oxygen concentration on COD concentration and bacterial growth

#### 4. Conclusion

Changing the operating conditions in aeration process can affect the aeration performance. Many conditions can be varied, one of them is oxygen concentration in air supply. From this research work it is shown that by supplying POME with air of three different oxygen concentration during aeration can:

- 1) Reduce the aeration time since oxygen mass transfer was increased.
- 2) Increase in oxygen concentration in air supplied as much as 6.7% in OEA and 80.1% in PO more than the DA can improved the DO concentration by reducing the percentage of DO deficit to 6.55% and 0.49% respectively on day 13.
- 3) Increased the microbial growth population to 15% and 18% more than growth experienced in POME aerated with DA.
- 4) The bacterial population does not directly affect the rate of oxygen transferred, instead affects the volume of oxygen transferred.

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